METAL COMPLEXES HAVING VITAMIN B_{12} AS A LIGAND

FIELD OF THE INVENTION

The invention relates to metal complexes that contain vitamin B_{12} as a ligand. The invention further relates to the use of these complexes in radiodiagnostics, radionuclide therapy, chemotherapy or as catalysts.

BACKGROUND ART

The current anti-cancer drugs, such as cisplatin, are also toxic to normal, healthy cells. The relatively high doses that need to be administered to a patient cause severe side effects. Enhanced selectivity by targeting cancer cells would be beneficial for the therapeutic index and the life quality of the patient.

In radionuclide therapy use is made of the metabolic accumulation of a radiopharmaceutical to deliver a therapeutic radiation dose to a tissue. The critical factor for successful radionuclide therapy is target tissue accumulation in relation to normal tissue, which is in the range of 5 to 100 in all methods known so far. An exception to this is the very successful iodine metabolic therapy of thyroid disease. Due to the low ratio of accumulation in target to normal tissue the radiation burden to the patient's normal tissues is often relatively high. A need thus exists for a way to specifically deliver the radionuclide to the target tissue.

An interesting candidate compound that may lead to site specific uptake is vitamin B_{12}. Fast proliferating cancer cells are so-called high B_{12} consumers. This very high demand makes vitamin B_{12} a potential "trojan horse" for delivering therapeutic agents.

Cyanocobalamin (vitamin B_{12}) is well known and its chemistry has been comprehensively reviewed. Many patents and publications exist for the derivatization of vitamin B_{12} at the cobalt, corrin ring or the ribose moiety. Some of these vitamin B_{12} derivatives have been proposed for application in cancer therapy or diagnosis but none have entered the market yet.

US2004/224921 for example relates to fluorescent cobalamins comprised of a fluorescent, phosphorescent, luminescent or light-producing compound that is covalently linked to cobalamin. These fluorescent cobalamins can be used as diagnostic and prognostic markers to distinguish cancer cells and tissues from healthy cells and tissues, and to determine if an individual will respond positively to chemotherapy using cobalamin-based therapeutic bioconjugates. The fluorescent, phosphorescent or light-producing compounds can be covalently linked to the cobalt atom, the corrin ring, or the ribose moiety of cobalamin. This type of derivatization is also described for non-fluorescent compounds.

Derivatization directly at the cobalt leads to a compound that retains more than 90% of the vitamin B_{12} activity. Derivatization at that position is thus an obvious choice. However, these compounds have also disadvantages. For example, cobalt alkylated compounds are light sensitive.

Derivatizations at the ribose or at positions on the corrin framework have the drawback of not being cleavable, thus influencing the biological behaviour of vitamin B_{12} significantly.

A need therefore exists for a drug which can be used for the diagnosis and treatment of cancer, which does not carry severe side effects nor leads to a high radiation burden.

SUMMARY OF THE INVENTION

It was found according to the invention that certain metal complexes are able to coordinate directly to the cyanide group in vitamin B_{12}. It could be shown that this type of binding is occurring in particular for the complex $[Tc(N\cap O)(OH_2)(CO)_3]$ ($N\cap O$ = bidentate ligand) in which the nitrogen atom of cyanide binds directly to the Tc metal centre forming a [Co]-CN-Tc moiety. This represents the prototypical example for a complex in which vitamin B12 acts as a ligand for a metal, in this case for Tc. However, other metal complexes in which vitamin B_{12} is a ligand that is linked to the metal through its cyanide are also part of this invention. In all these metal complexes vitamin B_{12} is acting as a ligand.

The invention for the first time proposes to coordinate vitamin B_{12} through its cyanide to a metal to form a [Co]-CN-M complex, wherein [Co] represents vitamin B_{12} without cyanide. The inventors have found that such vitamin B_{12} derivatives are chemically stable and can be easily produced.

With the exception of the CN position, all other sites in vitamin B_{12} have been proposed in the literature for labelling. The cyanide was not used before since it was not expected to act as a ligand group.

The invention thus relates to metal complexes of the general formula $M(L)_n$, wherein each L is independently selected nd is a ligand and at least one L is selected from vitamin B_{12} (cyanocobalamin) and its derivatives bound through the nitrogen atom of its cyanide group to M, which is an element selected from the transition metals, thus, forming a M-NC-[Co] moiety wherein [Co] represents vitamin B_{12} or its derivative without cyanide and wherein n is 1, 2, 3, 4, 5 to 6.

The different ligands L within one metal complex need not be the same but each L can be independently selected.

M can be a transition metal such as selected from technetium, ruthenium, rhodium, rhenium, palladium, platinum, iridium and copper.

For applications in cancer diagnosis and/or therapy, the metal is advantageously a radioisotope of Re or Tc, such as selected from ^{99m}Tc, ^{188}Re, ^{186}Re, ^{105}Rh.

When M is technetium or rhenium, the other ligands L are suitably three carbonyl groups (CO's) and optionally a bidentate ligand, optionally coupled to another metal, a biologically active molecule or another molecule, such as a fluorescent agent. It is also possible to have two monodentate ligands like H_2O or Cl instead of one bidentate ligand. Other monodentate ligands are monocarboxylate derivatives, mono thiolate derivatives, aliphatic/aromatic amines etc, optionally substituted with a biologically active molecule.

The bidentate ligand is suitably selected from two aliphatic and/or aromatic amine parts or one aliphatic or aromatic amine part and an anionic group such as a carboxylate, a thiolate or a hydroxylate. Examples are α-amino acids or derivatives of picolinic acid.

The ligand L may also be a biologically active molecule.

When M is platinum, L is selected from a ligand containing N, S, P O, C as the metal binding atom or any other donor with one non-binding electron pair available for coordination to the metal, optionally coupled to a biologically active molecule.

The biologically active molecule or other molecule is selected from fluorescing agents, pharmacophores with cytotoxic, cytostatic or other pharmacological activities,

optical dyes, NIR dyes or phosphorescent dyes (such as disclosed in US-6,180,085 and US-6,641,798). Fluorescing agents are for example fluoresceine, pyrene, acridine, dansyl or others. These can be used for diagnostic and prognostic purposes. Cytotoxic or cytostatic agents are for example tamoxifen, methotrexate and cyclophosphamid or other compounds with known pharmacological activity (for therapy). Alternatively, the other molecule may be a radioactive compound for diagnostic or therapeutic purposes. The biologically active molecule may also be a nucleic acid, such as RNA or DNA, in particular antisense RNA or DNA.

The invention relates further to a process for preparing these metal complexes containing vitamin B_{12} or a derivative thereof as a ligand, comprising mixing of vitamin B_{12} with a precursor complex, wherein M is a transition metal, n is 2, 3, 4, 5 or 6 and L is a ligand to obtain a metal complex with a stable [Co]-CN-M bridge. The precursor complex has the general formula $M(L)_{n-1}L'$, wherein L' is a ligand that is substituted by the vitamin B_{12}. The transition metal is suitably selected from technetium, ruthenium, rhodium, rhenium, palladium, platinum, iridium and copper.

The invention further relates to a precursor complex having the general formula $M(L)_{n-1}$, wherein M is a transition metal, preferably selected from technetium, ruthenium, rhodium, rhenium, palladium, platinum, iridium and copper, n is 2, 3, 4, 5 or 6 and L is a ligand.

The invention also relates to the metal complexes of the invention for use as a diagnostic or therapeutic agent, and to pharmaceutical compositions comprising the metal complexes of the invention.

Furthermore, the invention relates to metal complexes of the invention, wherein M is a metal with catalytic

activity, such as ruthenium, palladium, iridium, platinum or rhodium.

DETAILED DESCRIPTION OF THE INVENTION

The invention thus relates to metal complexes that contain vitamin B_{12} or one of its derivatives as a ligand. In one embodiment, the metal can be a radionuclide, such as ^{99m}Tc or ^{188}Re, for radiopharmaceutical application in e.g. cancer diagnosis and radionuclide therapy. In another embodiment, metal complexes obtained through coupling of other metal fragments (e.g. Rh, Pt, Pd) to vitamin B_{12} through the cyanide can be used for chemotherapy or stereospecific and/or enantioselective catalysis.

The reaction of vitamin B_{12} with metal complexes in a d^3, d^6 or d^8 electronic configuration leads to the formation of a stable [Co]-CN-M bridge. If M is ^{99m}Tc, this is a convenient method of labelling vitamin B_{12}. If M is e.g. Rh(I) the corresponding complex can be used for catalysis since vitamin B_{12} provides a stereospecific environment.

If M is ^{99m}Tc or ^{188}Re, then the precursor complex is suitably $[Tc(N\cap O)(OH_2)(CO)_3]$ in which OH_2 is substituted by [Co]-CN. The ligand N∩O (or other donor combinations) is variable. In the absence of other ligands (mono- or bidentate), only vitamin B_{12} is coordinated to the metal. In the absence of a bidentate N∩O ligand or a bidentate ligand of any other donor combination, one water is replaced by vitamin B_{12} and the two remaining positions at Tc remain occupied by H_2O or Cl. In solution, the complexes **b1-b4** with ^{99m}Tc and Re are received in quantitative yield although the isolated yield as a solid for Re was lower (see experimental part).

The ligand N∩O (or others) can also be bifunctional. One of the functions is used for coordination to the metal

and the second function can additionally be coupled to e.g. a targeting vector. This enables combination of receptor targeting, internalization and trapping of labelled vitamin B_{12}. Such a derivative is also called a "trojan horse" if the additional functionality is e.g. enzymatically cleaved inside the cell. It releases then a functionally active or inactive vitamin B_{12} compound.

Examples of bifunctional ligands are 1,4-dipicolinic acid, 1,5-dipicolinic acid, 1,2-imidazole dicarboxylic acid, 1,2-piperazine dicarboxylic acid, amino acids, like glutamic acid, lysine.

The structural and functional variability of the different complexes that can be attached to the cyanide of vitamin B_{12} thus offers the possibility to finetune and improve the general behaviour and bio-distribution of the [Co]-CN-$M(L)_{n-1}$ complex.

Vitamin B_{12} as a ligand exhibits unexpected stability in the complexes of the invention. It coordinates as native vitamin B_{12}. If it is released from the metal complexes of the invention by substitution with another ligand, it is released as native vitamin B_{12} and is, thus, not harmful to the body and safe.

When vitamin B_{12} coordinates to a toxic compound, its toxicity is expected to be reduced. It is known in the literature that certain enzymes such as adenosyl transferase cleave the cyanide or any other group attached to the cobalt in vitamin B_{12} from [Co]-CN. This means that also the metal complex $M(L)_{n-1}$ is cleaved and released from vitamin B_{12}. Upon release of the toxic moiety, which can be the metal complex or a molecule attached to the metal complex, in a cancer cell that has taken up the vitamin B_{12} complex the toxic moiety can perform its mode of action without inducing harmful side effects in non-targeted cells.

It was furthermore established that the kind of coupling via the cyanide as suggested by the invention does not affect binding of the vitamin B_{12} to transcobalamin I (TCI), transcobalamin II (TCII) and intrinsic factor (TF). It is thus still taken up by the cell and can thus successfully be used as a trojan horse.

The coupling of any other ligand L to M is via a binding atom selected from S, N, C, O, P or another atom containing one non-binding electron pair. The binding atom is thus part of a bigger moiety L.

The synthesis of the metal complexes of the invention is simple because there is no need of derivatization of the B_{12} framework. The products are readily available in very high yields. When a derivative of vitamin B_{12} is used as the ligand, derivatization of vitamin B_{12} can be performed before synthesis of the metal complex.

The compounds depicted in figures 1 and 2 are compounds of the invention.

To obtain the products **b1-b4**, the complex $[M(OH_2)(L^2)(CO)_3]$ (M= $^{185,187}Re$, ^{99m}Tc) was coordinated to vitamin B_{12}. The complex $[M(OH_2)(L^2)(CO)_3]$ is synthesized prior to the introduction of B_{12} reacting the *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex, respectively the $[ReBr_3(CO)_3]^{2-}$ complex, with a bidentate ligand L^2. This is the so-called mixed ligand [2+1]-approach. The bridging metal can be considered as a mediator between vitamin B_{12} and the ligand L^2, whereas the ligand L^2 is variable.

The diastereomers (a) and (b) of **b1-b4** can be separated from each other. The pure diastereomers are kinetically stable and interconvert, if ever, only slowly into each other. The distinct difference in HPLC retention time of the diastereoisomers indicates the possibility of using vitamin B_{12} as a chiral ligand for introducing enantio-

selectivity or diastereo-selectivity in reactions using metal complexes as catalysts.

In the metal complexes of the invention, vitamin B_{12} is not replaced by competing ligands, such as e.g. chloride, acetonitrile, water or other naturally occurring ligand groups.

Since light sensistivity in coenzyme B_{12} compounds containing Co-C (C≡alkyl) bond is related to the break of the Co-C bond, the complexes of the invention are not light sensitive at all. This is an important advantage as it simplifies storage and handling.

For use in diagnosis or radionuclide therapy, the subject metal complexes can be administered to the host, mostly a mammalian host, normally by injection, intravenously, intraarterially, peritoneally, intratumorally, or orally by means of a dosage form that will release the metal complex in the stomach. The route of administration depends upon the particular site at which the radionuclide is desired. Generally, from about 0.1 to 2 ml will be injected into a host, depending upon the weight of the host. Usually the treatment regime is tailor made because it depends on weight, type of tumor, age etc of the patient to be treated. The skilled person in the field is capable of determining the necessary radioactive dose. After administration of the radionuclide, depending upon its purpose, the host may be treated in various ways for diagnosis or therapy by detecting the radioactive emissions from the site or sites where the radionuclide specifically binds.

When the metal complexes are platinum complexes, they can be used in a pharmaceutical composition for chemotherapy. The route of administration is usually intravenous. Here again, the amount of metal complex that needs to be administered is determined for each patient separately.

The compound **b1** represents a typical example of a ^{99m}Tc derivative of vitamin B_{12}. It shows binding to the specific vitamin B_{12} transport proteins.

Compound **b2** is less lipohilic due to an additional carboxylic group in L^2 that is not involved in coordination to the $M(CO)_3$-moiety. The free carboxylic group can be considered as an anchor where any other biomolecule (e.g. peptide) or bioactive molecule or fluorescent marker can be attached to.

Compounds **b3** and **b4**, although obtained in lower yields (~30%), demonstrate the possibility to combine any type of artificial or natural α-amino acid with vitamin B_{12} via the [2+1]-approach.

Cytotoxicity tests of **b4** with a mouse melanoma cell line (B16F1) showed 17% of proliferation inhibition at a concentration of 100µM. At the same concentration, native cobalamin (vitamin B_{12}) showed no effect at all.

The cisplatinum compounds **b5-b7** demonstrate the possibility that vitamin B_{12} can also act as a ligand for metals different from Tc and Re. The chloride of complex **b5** is labile and can be replaced by a more stable ligand like e.g. methylguanine **b6** or guanosine **b7**. This offers the possibility to apply vitamin B_{12} as a trojan horse to deliver antisense RNA or DNA sequences into the cells and the cell nucleus for transcription or translation silencing.

Cytotoxicity tests with a mouse melanoma cell line (B16F1) showed a percentage of proliferative cells of 20% in case of **b5** and 30% in case of **b6**.

The present invention is illustrated in the Examples by reference to compounds in which the ligands form either a technetium or rhenium tricarbonyl, in which one of the OH_2 moieties is replaced by vitamin B_{12} and that may be further derivatized at the remaining OH_2 moieties, or a platinum

compound, such as cisplatin, in which one of the chloro atoms is replaced with vitamin B_{12} and the other chloro atom is optionally any other molecule.

However, the basic inventive idea is using the cyanide on vitamin B_{12} for derivatization. Based on this idea the skilled person is very well capable of defining other derivatives that are not as such disclosed herein but that still use the inventive idea. Such compounds are also part of this invention.

The invention is further illustrated in the non-limiting examples that follow, in which reference is made to the following figures.

Figure 1 shows precursor complexes before coupling to vitamin B_{12}. The numbering corresponds to the numbering used in the Examples.

Figure 2 shows examples of metal complexes of the invention. The numbering corresponds to the numbering used in the Examples.

Figure 3 shows an HPLC chromatogram of $^{99m}Tc(VitB_{12})(H_2O)(CO)_3$.

Figure 4 shows the structural formula of vitamin B_{12}.

Figure 5 shows X-ray structures of compounds **b1**, **b4**, **b5** and **b6**.

EXAMPLES

MATERIALS AND METHODS

All chemicals were purchased at highest commercial quality from Merck, Dietikon (CH), Aldrich or Fluka, Buchs (CH) and were used without further purification, unless stated otherwise.

All reactions were performed under nitrogen or argon atmosphere. Reactions were monitored by HPLC.

HPLC analyses were performed on a Merck-Hitachi L-7000 system equipped with a diode array UV/Vis detector and a EG&G Berthold LB 508 radiometric detector. Macherey Nagel Nucleosil C-18ec RP columns (5 µm particle size, 100Å pore size, 250x3 mm) and Merck C-18e RP Supersphere® columns (100Å pore size, 250x4 mm) and Waters XTerra RP8 columns (5 µm particle size, 3.0x100 mm) were used for separation. Different HPLC solvent systems and gradients were used: Solvent system 1: 0.1% AcOH and 10% CH_3CN in water, pH 3 (A) and methanol (B). Solvent system 2: 0.1% Triethylammonium acetate and 10% CH_3CN in water, pH 8 Solvent system 3: Trifluoroacetic acid, 0.1% in water (A) and methanol (B). Gradient 1: 0-3 min. 100% A, 3.1-9 min. 75% A, 9.1-20 min. 66% → 0% A, 20-25 min. 0% A ; 0.5 ml/min., or as mentioned. Gradient 2: 0-40 min. 100% → 0% A, 30.1-40 min. 0% A, 40.1-42 min. 0% → 100% A, 42-50 min. 100% A. Gradient 3: 0-5 min. 100% A, 5.1-40 min. 100% → 65% A, 40.1-45 min. 0% A, 45.1-53 min. 100% A. Gradient 4: 0-5 min. 0% → 20% B, 5-45 min. 20%.→ 65% B. Gradient 5: 0-10 min. 20% B, 10-30 min. 20% → 40% B. Gradient 6 : 0-30 min. 25% → 65% B. Gradient 7: 0-5 min. 25% B, 5.1-30 min. 25% → 100% B.

Preparative HPLC separations were performed on a Varian Prostar system equipped with two Prostar 215 pumps and a Prostar 320 UV/Vis detector, using Macherey Nagel Nucleosil C-18ec RP columns (7 µm particle size, 100 Å pore size, 250x20 mm, 10 ml/min. flow rate, and 250x40 mm, 40 ml/min. flow rate) and Waters XTerra Prep RP8 column (5 µm particle size, 100x30 mm, 30 ml/min. flow rate).

Electrospray ionization mass spectra (ESI-MS) were recorded on a Merck Hitachi M-8000 spectrometer.

UV/Vis spectra were recorded on a Varian Cary 50 spectrometer.

IR spectra were recorded on a Bio-Rad FTS-45 spectrometer with the samples in compressed KBr pills, unless mentioned differently.

RAMAN spectra were recorded on a Renishaw Ramanscope continuous wave instrument equipped with three lasers at 514 nm, 633 nm and 785 nm wavelength.

NMR spectra were recorded on a Varian Gemini 200 MHz or 300 MHz and a Bruker DRX 500 MHz spectrometer. The chemical shifts are reported relative to TMS using the residual solvent protons as internal standard. The chemical shifts of ^{31}P NMR spectra are reported relative to orthophosphoric acid at 0 ppm. The chemical shifts of ^{14}N NMR spectra are reported relative to nitromethane at 0 ppm. Peak assignments of cobalamin derivatives were determined by interpretation of the ^{1}H COESY, the C-H correlation, the DEPT and (in some cases) the ROESY spectra.

MALDI-ToF mass spectra were measured on a Voyager-DE PRO with a-cyano-4-hydroxycinnamic acid as matrix.
CV measurements were performed using a 757 VA Computrace Metrohm cyclo voltameter at room temperature using glassy carbon (Metrohm) as working electrode and auxiliary electrode, Ag/AgCl as reference electrode. The compounds were measured as a 1 mM solution in 0.1 M tetrabutylammonium hexafluorophosphate in methanol.

Crystallographic data were collected on a Stoe IPDS diffractometer at 183(2) K using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Suitable crystals were covered with Paratone N oil, mounted on top of a glass fibre and immediately transferred to a Stoe IPDS diffractometer. Data were collected at 183(2) K using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Eight thousand reflections distributed over the whole limiting sphere were selected by the program SELECT and used for unit cell parameter

refinement with the program CELL[1]. Data was corrected for Lorentz and polarisation effects as well as for absorption (numerical). Structures were solved with direct methods using SHELXS-97[2] or SIR97[3] and were refined by full-matrix least-squares methods on F2 with SHELXL-97[4].

Elemental analyses were performed on a Leco CHNS-932 elemental analyser.

$[NEt_3]_2[ReBr_3(CO)_3]$[5] and fac-$[^{99m}Tc(OH_2)_3(CO)_3]^+$[6] were prepared as previously reported.

GENERAL LABELLING PROCEDURES

A 10 ml glass vial with rubber stopper was flushed with dinitrogen. 50 ml of a 10^{-3} M aqueous cobalamin derivative or ligand containing solution and 450 ml of a 0.1 M phosphate buffered $[^{99m}Tc(OH_2)_3(CO)_3]^+$ solution (pH 7.4) were added and the reaction mixture was kept at 75°C for 30-45 minutes.

The cyanide bridged cobalamin derivatives were prepared as follows: 50 ml of a 10^{-3} M aqueous soln. of bidentate ligand and 450 ml of phosphate buffered $[^{99m}Tc(OH_2)_3(CO)_3]^+$ solution (pH 7.4) were added to a dinitrogen flushed vial (as described above) and the reaction mixture was kept at 90°C for 30 minutes. 100 ml of this soln. were added to another vial, containing 100 ml of 10^{-2} M aq. vitamin B_{12} solution. This reaction mixture was kept at 37°C for 60 minutes.

HPLC analysis with γ-detection was performed to verify full conversion of the ^{99m}Tc species.

Synthesis of $[Re(imc)(OH_2)(CO)_3]$ (1)

$(NEt_4)_2[ReBr_3(CO)_3]$ (488 mg, 0.63 mmol) was dissolved in water (5 ml) and added to a solution of 4-imidazole carboxylic acid (imc) (71 mg, 0.63 mmol) in water (3 ml).

After 2 h under reflux, the product precipitated as a white powder at r. t. Filtration, washing with diethyl ether and drying at high vacuum gave 186 mg of **1** (73%).

^{1}H NMR (300, DMSO-D6) δ 8.40 (*s*, 1H, imc), 7.68 (*s*, 1H, imc), 7.08 (*br s*, 1H, imc)

IR (KBr, cm^{-1}): 2039, 1936 $\nu_{C=O}$ (st)

MS (ESI+, MeOH) m/z: 764 (2M - $2H_2O$), 382 (M - H_2O)$^+$

Anal. Calcd for $C_7H_5N_2O_6Re$: C, 21.05; H, 1.26; N, 7.02 Found: C, 21.15; H, 1.41; N, 6.97

Synthesis of [Re(2,4-dipic)(OH_2)(CO)3] (2)

$(NEt_4)_2[ReBr_3(CO)_3]$ (101 mg, 0.13 mmol) was dissolved in water (10 ml). After addition of $AgNO_3$ (70 mg, 0.4 mmol) and stirring at r. t. for 3 h, AgBr was removed by filtration and pyridine-2,4-dicarboxylic acid (2,4-dipic) (24 mg, 0.13 mmol) was added to the colourless solution, followed by stirring at 50°C for 2 h. The now yellow solution was cooled down and the product was allowed to crystallize at 4°C for 12 h. The yellow crystals were collected by filtration, washed with water and dried at high vacuum to give the product in a yield of 38 mg (65%).

^{1}H NMR (300, DMSO-D6) δ 8.96 (*d*, 1H, pyridine), 8.34 (*s*, 1H, pyridine), 8.15 (*d*, 1H, pyridine), 7.50 (*s*, 1H, pyridine)

IR (KBr, cm^{-1}): 2036, 1920 $\nu_{C=O}$ (st)

MS (ESI+, MeOH) m/z: 438 (M - H_2O)$^+$

Anal. Calcd for $C_{10}H_6NO_8Re$: C, 26.45; H, 1.33; N, 3.07 Found: C, 26.15; H, 1.67; N, 3.07

Synthesis of [^{99m}Tc (ser)(OH2)(CO)3] (3)

Complex **3** was prepared according to the standard labelling procedure described in the chapter 'Materials and

Methods'. Serine was labelled with a concentration of 10^{-3} M in the r.m. The ^{99m}Tc was completely converted after 40 min. at 90°C.

Synthesis of [Re(dmg)(OH_2)$(CO)_3$] (4)

(Et4N)2[ReBr3(CO)3] (100 mg, 0.13 mmol) was dissolved in a methanol/water mixture (4:1, 10 ml). N,N-dimethylglycine (70 mg, 0.7 mmol) was added and the mixture was stirred for 12 h at 50°C. The solution was allowed to equilibrate to room temperature, concentrated and purified on a short C18 filter. A white crystalline solid was obtained. Yield: 20 mg (40%).

^1H NMR (500, DMSO-D6) δ 4.18 (*s*, 2H), 3.46 (*s*, 3H), 3.15 (*s*, 3H)

Anal. Calcd for $C_{21}H_{24}N_3O_{15}Re_3$ (trimer): C, 22.58; H, 2.17; N, 3.76 Found: C, 23.19; H, 2.78; N, 3.84

IR (KBr, cm^{-1}): 2022, 1911, 1890, 1866

MS (ESI+, MeOH) m/z: 1117.0 ([M]+) (trimer)

Synthesis of Compound (b1)

A solution of vitamin B_{12} (100 mg, 73.8 mmol) and **1** (50 mg, 0.125 mmol) in 10 ml of methanol was stirred at room temperature over night. The reaction was controlled by HPLC measurements (solvent system 1, gradient 2). When no vitamin B_{12} was detectable anymore, the solvent was evaporated, the crude product redissolved in water and filtered over a 0.2 µm filter to remove excess of **1**. The filtrate was subjected to preparative HPLC purification (solvent system 1, gradient 2, 30 ml/min flow). The two isomers were separated from each other. The isomers were obtained as red powder in similar amounts ~(1:1). Total yield: 96%. For full characterisation see crystallographic data. Crystals of **b1(b)** were obtained by vapor diffusion of acetone into an aqueous solution of **b1(b)**.

^{31}P NMR (81, CD_3OD) δ 0.68

IR (KBr, cm^{-1}): 3400, 2926, 2179, 2023, 1900, 1667, 1627, 1572, 1401, 1366, 1213, 1056

MS (ESI+, MeOH) m/z: 1737 $(M + 1)^+$, 869 $(M + 2)^{2+}$

CV: $E_{1/2}$ = -652 mV vs. Ag+/AgCl, ca. 90% reversible

Synthesis of Compound (b2)

A solution of vitamin B_{12} (50 mg, 36.9 mmol) and **2** (19.8 mg, 0.074 mmol) in 3 ml of methanol was stirred at room temperature for 48 hrs. The reaction was controlled by HPLC measurements (solvent system 1, gradient 2). When no vitamin B_{12} was detectable anymore, the solvent was evaporated and the crude product subjected to preparative HPLC purification (solvent system 1, gradient 2, 30 ml/min flow). The isomers were obtained separately as red powder in similar amounts ~(1:1). Total yield: 76%

^1H NMR (500, D_2O) δ 0.43 (*s*, 3H, C20), 0.95-0.98 (*m*, 1H, C41'), 0.99 (*s*, 3H, C46), 1.08 (*s*, 3H, C54), 1.17-1.20 (*m*, 1H, C60'), 1.23 (*d*, 3H, Pr3, J = 6.3 Hz), 1.42 (*s*, 3H, C36), 1.44 (*s*, 3H, C47), 1.65-1.80 (*m*, 2H, C42', C48'), 1.83 (*s*, 3H, C25), 1.88-2.10 (*m*, 6H, C30, C37, C41, C42, C48), 2.25 (*s*, 3H, B10), 2.28 (*s*, 3H, B11), 2.37-2.38 (*m*, 2H, C26), 2.41 (*s*, 3H, C53), 2.45-2.53 (*m*, 6H, C31, C49, C55), 2.56 (*s*, 3H, C35), 2.58-2.65 (*m*, 3H, C56, C60), 2.75-2.81 (*m*, 3H, C18, C37, Pr1'), 3.15 (*d*, 1H, C13, J = 9.9 Hz), 3.63 (*d*, 1H, Pr1, J = 13.7 Hz), 3.70 (*dd*, 1H, R5', J = 12.6 and 4.2 Hz), 3.78 (*d*, 1H, C19, J = 11.4 Hz), 3.86-3.90 (*m*, 2H, C8, R5), 4.00-4.02 (*m*, 1H, R4), 4.15 (*t*, 1H, R2, J = 4.2 and 4.1 Hz), 4.26-4.33 (*m*, 1H, Pr2), 4.39 (*d*, 1H, C3, J = 8.6 Hz), 4.63 (*dt*, 1H, R3, J = 8.5 and 4.3, 4.0 and 3.6 Hz), 6.08 (*s*, 1H, C10), 6.23 (*d*, 1H, R1, J = 3.0 Hz), 6.51 (*s*, 1H, B4), 7.02

(*s*, 1H, B2), 7.24 (*s*, 1H, B7), 8.13 (dd, 1H, L3, J = 5.4 and 1.7 Hz), 8.51 (s, 1H, L1), 8.71 (*d*, 1H, L2, J = 5.4 Hz)

^{13}C NMR (125, D_2O) δ 15.8, 16.5, 17.5, 18.4, 19.7, 19.9, 20.3, 20.4, 20.9, 21.1, 27.6, 27.7, 29.5, 32.4 (3C), 33.1 (2C), 33.7, 35.2, 36.3, 40.2, 42.8, 43.9, 46.9, 48.2-52.6 (2C below the solvent signal), 52.6, 55.3, 55.8, 58.4, 60.6, 62.7, 70.8, 73.7, 75.5, 76.9, 83.9, 86.7, 88.3, 96.1, 105.1, 108.1, 112.9, 117.6, 128.0, 129.6, 131.5, 134.3, 136.1, 138.0, 143.3, 152.0, 154.4, 166.4, 167.1, 168.0, 174.2 (3C), 175.3 (2C), 176.6 (3C), 177.6 (2C), 178.2, 180.7, 182.5, 193.4, 196.3 (2C)

MS (ESI+, MeOH) *m/z*: 1793 $(M + 1)^+$, 1116 (fragment)

IR (KBr, cm^{-1}): 3411, 2972, 2179, 2027, 1918, 1903, 1665, 1611, 1572, 1498, 1402, 1214, 1154, 1061, 571

Synthesis of compound (b3) with M=^{99m}Tc

500 ml of a 10^{-4} M PBS soln. of **3**, pH 7.4 were mixed with 500 ml of a 0.01 M aqueous soln. of vitamin B_{12} and stirred at 40°C for 1.5 hrs. The reaction was followed by HPLC (solvent system1, gradient 2). The reaction reached an equilibrium after a turnover of 60%.

Synthesis of Compound (b4)

Vitamin B_{12} (50 mg, 0.04 mmol) was dissolved in methanol (10 ml). $[Re(dmg)(CO)_3]_3$ **4** (45 mg, 0.04 mmol) was added and the mixture was stirred at r.t. for 12 h. Two adducts (clearly distinguishable by HPLC) formed (yield 25% and 36%). These were isolated and purified by preparative HPLC (solvent system 3, gradient 7). Yield: 10 mg, 14% (adduct 1), 12 mg, 17% (adduct 2). Crystals of **b4(b)** suitable for x-ray analysis were obtained by vapour diffusion of

acetone in a H_2O solution of the complex. For full characterization see crystallographic data.

Anal. Calcd for $C_{70}H_{96}CoN_{15}O_{19}PRe$: C, 48.66; H, 5.60; N, 12.16
Found: C, 48.42; H, 5.01; N, 12.04 **(b4(b))**
IR (KBr, cm^{-1}): 2033, 1928, 1904
MS (ESI+, MeOH) *m/z*: 1728.7 $(M + 1)^+$

Synthesis of Compound (b5)

A mixture of *cis*-diamminedichloroplatinum(II) **(5)** (66.4 mg, 0.221 mmol) and silver nitrate (37.6 mg, 0.221 mmol) in water (6 ml) was stirred at 35°C for 2 hours. The precipitation was removed by centrifugation and washed with water (4 ml). The solutions were added to cyanocobalamin (300 mg, 0.221 mmol), and the resulting solution was stirred at 50°C for 16 hours. HPLC analysis exhibited full conversion of the cobalamin. The solvent was removed *in vacuo*, and the crude product was purified by preparative HPLC (solvent system 3, gradient 4). Lyophilization of the product fraction gave **b5** as a red powder. Yield: 259.8 mg, 72.6%.
Crystals of **b5** were obtained by vapour diffusion of acetone into a saturated aqueous solution of **b5**.
UV/Vis: λ/nm (log ε/mol $l^{-1}cm^{-1}$) = 279.9 (4.1), 361.9 (4.4), 519.9 (3.8), 550.9 (3.8).
IR (KBr, cm^{-1}): ν_{CN} (st) 2199
^{195}Pt NMR (107, CD_3OD) δ -2340
MALDI-ToF MS *m/z*: 1607 $[M - Cl + Na]^+$, 1591 $[M-Cl-NH_3+Na]^+$, 1571 $[M - Cl - 2NH_3 + Na]^+$
CV: $E_{1/2}$ = -515 mV vs. Ag+/AgCl, ca. 50% reversible

Synthesis of Compound (b6)

A solution of **b5** (37.4 mg, 23.1 µmol) and 9-methylguanine **(6)** (4.2 mg, 25 µmol) in water (2 ml) was

stirred at 50°C. After 4 days, HPLC analysis showed almost complete conversion of the starting materials. The solvent was removed *in vacuo*, and the crude product was purified by preparative HPLC (solvent system 3, gradient 5). Lyophilization of the product fraction gave **b6** as a red powder. Yield: 32.0 mg, 79%. Crystals of b6 were obtained by vapour diffusion of acetone into a saturated aqueous solution of **b6**.

^{31}P NMR (202, CD_3OD): δ 0.73

MALDI-ToF MS: 1736 $[M - CH_3]^+$, 1715 $[M - NH_3 - CH_3]^+$

Synthesis of Compound (b7)

A solution of **b5** (58.5 mg, 36.1 µmol) and 2'-deoxyguanosine **(7)** (11.6 mg, 43.3 µmol) in water (5 ml) was stirred at 30°C. After 4 days, HPLC analysis showed almost complete conversion of the starting materials. The solvent was removed *in vacuo*, and the crude product was purified by preparative HPLC (solvent system 3, gradient 6). Lyophilization of the product fraction gave **b7** as a red powder. Yield: 45.3 mg, 67.7%.

UV/Vis: λ/nm (log ε/mol $l^{-1}cm^{-1}$) = 278.0 (4.2), 361.9 (4.2), 521.0 (3.7), 546.0 (3.7).

^{31}P NMR (202, CD_3OD): δ 0.71 (94%), 0.21 (6%)

^{195}Pt NMR (107, CD_3OD): δ -2475 (line with ca. 1.5 kHz)

MALDI-ToF MS: 1723 [M - ribose - $2NH_3$ + Na]$^+$.

Preparation of $^{99m}Tc(VitB_{12})(X)_2(CO)_3$ [X = Cl, H_2O]

$^{99m}Tc(H_2O)_3(CO)_3^+$ is prepared by adding 1 ml (50 mCi, but the radioactivity concentration does not need to be 50 mCi/ml, it works with lower and with higher) ^{99m}Tc-pertechnetate to an IsoLink™ vial and boiling the resulting solution for 20 minutes. The tricarbonyl reaction mixture is allowed to cool down and acidified to pH 3 with HCl 1N.

Then, a 10 mM aqueous solution of vitamin B_{12} is prepared by dissolving 68.0 mg of the vitamin in 5 ml of oxygen-free Water for Injections. A mixture of 0.1 ml of the ^{99m}Tc-tricarbonyl solution and 0.9 ml of the 10 mM vitamin B_{12} solution is allowed to react at 100°C for 30 minutes under nitrogen atmosphere.

Preparation of $^{186}Re(VitB_{12})(X)_2(CO)_3$ [X = Cl, H_2O]

A previously acidified (pH 2.5) solution of Re-186-perrhenate is added to a vial, sealed under nitrogen atmosphere, which contains a mixture of NH_3BH_3 and ascorbic acid. The reaction is completed after 10 minutes at room temperature. This is called a pre-reduction step.

A 10 mM aqueous solution of vitamin B_{12} is prepared by dissolving 68.0 mg of the vitamin B_{12} in 5 ml of oxygen-free Water for Injections. From the reduced rhenium solution, 1 ml is added to an IsoLink™ vial and allowed to react for 15 minutes at 100°C. The resulting solution, containing $^{186}Re(H_2O)_3(CO)^{3+}$, is cooled down.

A mixture of 0.1 ml of the Rhenium tricarbonyl solution and 0.9 ml of the Vitamin B12 10 mM solution is kept at 100°C for 45 min.

Figure 3 is an example of an HPLC chromatogram of $^{99m}Tc(Vit\ B_{12})(H_2O)_2(CO)_3$.

CRYSTALLOGRAPHIC DATA

X-Ray Table of Compound b1(b)

Empirical formula	C70 H106 Co N16 O28 P Re
Formula weight	1895.81
Temperature	183(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P212121
Unit cell dimensions	a = 15.9578(10) Å α= 90°.
	b = 21.2328(12) Å β= 90°.
	c = 27.9776(13) Å γ= 90°.
Volume	9479.6(9) Å3
Z	4
Density (calculated)	1.328 Mg/m³
Absorption coefficient	1.545 mm⁻¹

F(000)	3916
Crystal size	0.57 x 0.15 x 0.04 mm³
Crystal description	red plate
Theta range for data collection	2.16 to 25.00°.
Index ranges	-18<=h<=18, 0<=k<=25, 0<=l<=32
Reflections collected	16557
Independent reflections	16557 [R(int) = 0.0000]
Reflections observed	10604
Criterion for observation	>2sigma(I)
Completeness to theta = 25.00°	98.8 %
Absorption correction	Numerical
Max. and min. transmission	0.9331 and 0.5508
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	16557 / 40 / 1026
Goodness-of-fit on F2	0.954
Final R indices [I>2sigma(I)]	R1 = 0.0990, wR2 = 0.2370
R indices (all data)	R1 = 0.1297, wR2 = 0.2549
Absolute structure parameter	-0.007(11)
Largest diff. peak and hole	1.975 and -0.854 e.Å^{-3}

X-Ray Table of Compound b4(b)

Empirical formula	C73 H112.60 Co N15 O27.30 P Re
Formula weight	1913.28
Temperature	183(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P212121
Unit cell dimensions	a = 15.8758(7) Å α= 90°.
	b = 21.8451(10) Å β= 90°.
	c = 26.3673(14) Å γ= 90°.
Volume	9144.4(8) Å³
Z	4
Density (calculated)	1.390 Mg/m³
Absorption coefficient	1.601 mm^{-1}
F(000)	3964
Crystal size	0.46 x 0.08 x 0.07 mm³
Theta range for data collection	1.86 to 26.00°.
Index ranges	-19<=h<=19, -26<=k<=26, -32<=l<=32

Reflections collected	68628
Independent reflections	17853 [R(int) = 0.1048]
Completeness to theta = 26.00°	99.5 %
Absorption correction	Numerical
Max. and min. transmission	0.9257 and 0.7060
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	17853 / 2 / 1071
Goodness-of-fit on F2	0.900
Final R indices [I>2sigma(I)]	R1 = 0.0662, wR2 = 0.1588
R indices (all data)	R1 = 0.1159, wR2 = 0.1742
Absolute structure parameter	-0.014(8)
Largest diff. peak and hole	2.044 and -1.063 e.Å^{-3}

X-Ray Data and Structure Refinement of Compounds b5

Empirical formula	$C_{137}H_{184}C_{12}Co_2F_3N_{32}O_{56}P_2Pt_2$
Formula weight	3873.04
Temperature	183(2) K
Wavelength	0.71073 Å
Crystal system	P1
Space group	Triclinic
Unit cell dimensions	a = 16.9434(17) Å, α = 111.999(10)°. b = 17.3115(15) Å, β = 99.721(11)°. c = 18.0814(17) Å, γ = 90.580(11)°.
Volume	4831.2(8) Å^3
Z	1
Density (calculated)	1.331 Mg/m^3
Absorption coefficient	1.741 mm-1
F(000)	1979
Crystal size	0.34 x 0.14 x 0.10 mm^3
Theta range for data collection	2.31 to 28.05°.
Index ranges	-22<=h<=22, -22<=k<=22, -23<=l<=23
Reflections collected	46915
Independent reflections	38480 [R(int) = 0.0685]
Completeness to theta = 28.05°	92.0 %
Max. and min. transmission	0.8545 and 0.7101
Refinement method	Full-matrix least-squares on F^2

Data / restraints / parameters	38480 / 61 / 1937
Goodness-of-fit on F2	0.914
Final R indices [I>2sigma(I)]	R1 = 0.0720, wR2 = 0.1731
R indices (all data)	R1 = 0.1138, wR2 = 0.1925
Absolute structure parameter	-0.015(5)
Largest diff. peak and hole	1.141 and -1.967 e.Å^{-3}

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